

Национальная академия наук Украины  
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## *Pontus Euxinus 2011*

по проблемам водных экосистем,  
посвящённой 140-летию Института биологии южных морей  
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*Protopteridinium divergens* (Ehr.) Balech. Неблагоприятная обстановка по данным флористических исследований сложилась в районах выхода сточных вод, где концентрация питательных веществ, вероятно, намного превышающая показатели остальной акватории порта, способствовала массовому развитию эвгленовых *Eutreptia lanowii* Steur. (48,3 млн. кл/м<sup>3</sup>) и сине-зеленых водоросли рода *Lyngbya* (до 1 млн.кл/м<sup>3</sup>), приуроченных обычно к поли- и мезотрофным участкам моря и являющихся обильным компонентом планктона прибрежных акваторий, подверженных наибольшему антропогенному загрязнению (Нестерова, 1986).

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**NOVEL METHODOLOGY FOR IDENTIFYING ALIVE VERSUS DEAD COPEPODS IN MARINE ENVIRONMENTS**

Further progress in studying marine zooplankton mortality depends on developing new, improved approaches of differentiation between live and dead organisms. Staining freshly collected samples of zooplankton with a vital dye still remains the optimal solution of the problem. Fluorescein diacetate (FDA) has been widely used in algal and bacterial viability assays but, surprisingly, no attempts were made to apply it in zooplankton studies, with the exception of an embryo viability assay in a culture of the copepod *Calanus helgolandicus* (Buttino *et al.*, 2004). In this study, we tested how efficient FDA is for staining larval stages and adult marine copepods from a culture and a natural community. In a set of experiments with a model culture of the copepod *Calanipeda aquae dulcis*, an accurate differentiation between alive and killed (formaldehyde fixation or heat shock) specimens was achieved after staining them with FDA and neutral red (NR) as a control dye. The FDA staining protocol and

epifluorescence microscopy were then successfully applied for identifying alive versus dead copepods in a coastal marine ecosystem (Sevastopol bay, the Black Sea), with the potential the new marker has in a study of other zooplankton taxa being investigated.

Irrespective of the dye nature, the assay accuracy depends to a large extent on the dye colour intensity and the subjective assessment of the person who is performing classification of the organisms to live and dead. As against NR, FDA provided much better hue/brightness contrast between the dead (unstained) and live (stained) specimens. To avoid any subjectivity in the classification, we have improved it by: (i) measuring average colours (RGB and/or HSB models) of the organisms, using their digital images and an original image editing software; (ii) classifying the organisms visually to the three classes, Live (L), Dead (D) and Questionable (Q), depending on their colour/brightness; (iii) applying stepwise discriminant analysis to reduce the data dimensionality and build the classification model, with the classes L and D serving as the training set; (iv) classifying the organisms from the pool Q to the classes L or D, using the above discrimination model. This made the analysis highly accurate and subjectivity-free. The new methodological approach involving the FDA-staining protocol and the discriminant analysis appears to be a promising tool for studying zooplankton mortality in the sea.

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#### **MICRO-SPATIAL STRUCTURE OF COMMUNITIES OF HETEROTROPHIC FLAGELLATES (PROTISTA) FROM A SPHAGNUM BOG**

Spatial distribution pattern of heterotrophic flagellates within a macroscopically homogenous sphagnum parcel of a transitional bog in the southern taiga was studied. Under investigation was horizontal pattern at different scales (1 cm, 10 cm, 1 m, 10 m) and the vertical heterogeneity of the community in the sphagnum quagmire. 105 species and forms of heterotrophic flagellates were revealed. Predominating were euglenids, less abundant are kinetoplastids and cercomonads. The most numerous appeared to be *Cryptomonas* sp.; *Bodomorpha minima* Hollande, 1942; *Goniomonas truncata* (Fresenius, 1858) Stein, 1887; *Protaspis simplex* Vørs, 1992; *Bodo designis* Skuja, 1948; *B. saltans* Ehrenberg, 1832; *Phyllomitus apiculatus* Skuja, 1948; *Paraphysomonas* sp.; *Petalomonas minuta* Hollande, 1942. More abundant species were characterized by less patchy distribution than less abundant. At a